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A Standardized Nomenclature for Endogenous Mouse Mammary Tumor Viruses

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We propose a revised standardized nomenclature for endogenous mouse mammary tumor viruses based on characterization by molecular cloning techniques and genetic segregation data.

Within the last 5 years, studies on the endogenous mouse mammary tumor viruses (MMTVs) have greatly expanded our knowledge of the different proviral genes in inbred and feral mice. However, the absence of generally agreed-upon designations for these genes has created some confusion in the literature. Problems arise in naming newly defined genes as well as in referring to known genes with as many as six different designations. Furthermore, the accurate identification of discrete loci has been hampered by several factors.

(i) The conventional use of the restriction enzyme *Eco*RI has complicated the characterization of individual loci, because this enzyme produces two cell-virus junction fragments for each full-length provirus. Early attempts to pair 3′ and 5′ fragments derived from individual loci used representative cDNA probes which failed to distinguish the 3′ and 5′ proviral ends by hybridization. Furthermore, these studies were based largely on comparisons among inbred strains of mice, often without the support of clear-cut genetic segregation data.

(ii) While there are generally few endogenous MMTVs per haploid genome, a number of different proviral genomes produce cell-virus junction fragments of approximately equivalent size which are not always distinguishable on Southern blots.

Because of inconsistencies which have become established in the literature, we have jointly agreed upon a revised nomenclature for the endogenous MMTVs. The nomenclature we describe here is based on the following guidelines.

(i) It is in agreement with the nomenclature rules established for mouse genes (Committee on Standardized Genetic Nomenclature for Mice, Mouse Newsl. **72**:2–21, 1985) and is basically an elaboration of the *Mtv-* numerical system currently favored by most researchers in the field.

(ii) It includes only proviral loci which have been described in published studies and which have been molecularly cloned and characterized or which have been clearly distinguished from other proviruses by chromosomal mapping or independent assortment in classical segregation analysis.

MMTV proviral genes. Table 1 shows the proviral genes with their known chromosomal locations and inbred strain distributions. Table 2 shows the commonly used inbred strains and all of the MMTV proviral loci they contain. The *Eco*RI fragments which define each provirus have been identified by using data from genetic studies with hybrid mice or somatic cell hybrids or, alternatively, from strain comparisons with flanking sequence probes. There are considerable variations in the size estimates made by different laboratories in referring to the same *Eco*RI fragments, depending to a large extent on the standards used and the efforts taken to avoid electrophoretic anomalies. The sizes given in Table 1 for each fragment are based on the commonest usage in the literature, and the numbers should not be regarded as true sizes.

Briefly, the *Mtv* proviruses included in Table 1 are as follows.

Mtv-1, *Mtv-2*, and *Mtv-3*. These were the first endogenous MMTVs to be identified and were originally described by their association with virus gene expression and tumorigenesis. *Mtv-1* is expressed as an infectious virus and is associated with late-occurring tumors (26, 28). The expression of virus from *Mtv-2* is also associated with tumors, but these

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TABLE 1. Characteristics and chromosomal locations of MMTV proviral loci

Genetic designation	Former designation(s)	Size (kb) of *Eco*RI fragments (reactivity with MMTV probes)	Chromosomal location	Mouse strains[a]	References
Mtv-1	Unit V	6.5 (5′) 4.5 (3′)	7	DBA, C3H/He, C3H/An, C3H/Se[b]	10, 17, 24, 26, 28
Mtv-2		6.9 (5′) 11.0 (3′)	18	GRS	11, 27
Mtv-3	*Mtv-18*	17.4 (5′) 6.9 (3′) 0.9[c]	11	GRS	9, 11, 13
Mtv-6	Unit I *Mtv-19*	16.7	16	BALB/c, CBA, A, C3H, DBA	1, 2, 5, 9, 14, 15, 20, 24
Mtv-7	Unit Ia Unit IV *Mtv-19* *Mtv-10*	16.7 (5′) 11.7 (3′)	1	C3H/Bi, C3H/Ki, C3H/Sm[d], DBA, NFS, GRS	5, 9, 11, 18, 24
Mtv-8	Unit II *Mtv-16* GR40	7.8 (5′) 6.7 (3′)	6	DBA/2, BALB/c, STS/A, RIII, CBA, AKR, A, BR6, GRS, C3H, NFS, C57BL	2, 3, 5, 6, 12, 15, 24
Mtv-9	Unit III	7.8 (5′) 10.0 (3′)	12	BALB/c, CBA, C57BL, AKR, NZB, C3H/Bi, C3H/Ki, C3H/Sm[d]	1, 2, 15, 18, 23
Mtv-11	Unit VII Unit VI *Mtv-7a* *Mtv-12* Unit Ib	15.0 (5′) 5.8 (3′)	14	DBA/2, C3H/He, C3H/An, C3H/Se[b]	5, 9, 17, 19, 20
Mtv-13	Unit VIII Unit VII *Mtv-11*	9.0 (5′) 5.8 (3′)	4	DBA/2, NFS, A	5, 9, 12
Mtv-14	Unit IX	1.7		DBA/2, C3H, GRS,CBA, CE, NZB, NFS	5, 9, 11, 20, 24
Mtv-17	Unit IV *Mtv-10* Unit XI *Mtv-20* *Mtv-15*	10.0 (5′) 8.3 (3′)	4	DBA/2, C57BL, AKR, NZB, BR6, C3H/Bi, C3H/Ki, C3H/Sm[d], GRS, NFS, DDSio, C58	5, 9, 11, 15, 16, 18; J.-N. Yang, R. T. Boyd, P. D. Gottlieb, and J. P. Dudley, Immunogenetics, in press.
Mtv-20	Unit B	13.0 5.3		C57BL[e]	25
Mtv-21		8.0 (5′) 8.1 (3′)	8	BR6	15

[a] Substrains are listed only where differences exist.
[b] Previously C3H/HeSed.
[c] Internal fragment reactive with 3′ *env* sequences.
[d] Previously C3H/StWi.
[e] Not in C57BL/10 or C57BL/6 nor in all colonies of C57BL.

occur early in life and are pregnancy dependent (27). The provirus at *Mtv-3* does not produce infectious virus, only internal virion proteins (13). Molecular techniques have now identified the proviral sequences at these sites (2, 10, 11, 24).

Mtv-6. This provirus was originally identified as endogenous Unit I and was clearly shown to be subgenomic despite residing on a 16.7-kilobase (kb) *Eco*RI fragment (2). This fragment maps to chromosome 16 and hybridizes strongly with a long-terminal-repeat probe (1). It is also reported to hybridize weakly with *env* and *gag* probes but not at all with a *pol* probe (14, 15). Some workers have suggested that *Mtv-6* may be a reverse transcript of, or the template for, a subgenomic mRNA from the open reading frame in the MMTV long terminal repeat (1).

Major confusion has arisen over this locus. At least two other proviral genes, *Mtv-7* and *Mtv-11*, yield comigrating or closely migrating *Eco*RI fragments (see below), and it has also been suggested that some mouse strains contain additional proviruses which generate 15- to 17-kb fragments. For example, for BALB/c mice, which lack both *Mtv-7* and *Mtv-11*, some workers have reported a doublet in this size range reactive with viral long-terminal-repeat sequences (4).

TABLE 2. MMTV proviral loci of common inbred strains

Strain	*Mtv* proviruses
A/St	*Mtv-6, -8, -13, -23*[a]
AKR	*Mtv-8, -9, -17, -22*[a], *-23*[a]
BALB/c	*Mtv-6, -8, -9*
BR6	*Mtv-8, -17, -21*
CBA	*Mtv-6, -8, -9, -14*
C3H/He, C3H/An, C3H/Se	*Mtv-1, -6, -8, -11, -14*
C3H/Bi, C3H/Ki, C3H/Sm	*Mtv-6, -7, -8, -9, -14, -17*
C57BL/6, C57BL/10	*Mtv-8, -9, -17*
C57BL	*Mtv-8, -9, -17, -20*[b]
DBA/2	*Mtv-1, -6, -7, -8, -11, -13, -14, -17*
GRS, STS	*Mtv-2, -3, -7, -8, -14, 17*
NFS	*Mtv-7, -8, -13, -14, -17, ?*[c]
NZB	*Mtv-9, -17, -22*[a], *-24*[a]

[a] Provisional.
[b] Not in all C57BL mice.
[c] Some but not all NFS mice contain at least one additional proviral locus detected in *Bam*HI digests, but the corresponding *Eco*RI fragment(s) comigrates with other bands (5).

However, there is no other evidence that BALB/c mice contain a second provirus producing a fragment of this size. Other researchers have speculated that a discrete proviral gene generating a 16.7-kb fragment is present in C3H/He mice (19). However, since this proviral locus has not been shown to be distinct from the *Mtv-11* provirus also carried by C3H mice, it is not presently listed as a separate proviral locus.

Mtv-7 is composed of a 5′ 16.7-kb and a 3′ 11.7-kb fragment (5, 10, 15, 18). It is located on chromosome 1 (9, 11, 24). Previous studies had mapped a 16.7-kb fragment to chromosome 1 as *Mtv-7*, and the 11.7-kb fragment was formerly thought to be associated with a 10-kb fragment in a locus designated *Mtv-10* and also mapped to chromosome 1 (24). However, this 10-kb fragment is now known to be derived from the locus designated *Mtv-17* (see below), and it is now clear that chromosome 1 contains only a single proviral genome, *Mtv-7*.

Mtv-8, previously referred to as Unit II (2), is probably the best-characterized endogenous MMTV provirus. It is carried by most inbred strains, and it has been cloned by several investigators and analyzed by transfection and DNA sequencing (6, 20, 21). From the strain distribution and restriction mapping data, it also appears that *Mtv-8* is identical to a locus designated *Mtv-16* or GR40 in previous reports (6, 23).

Mtv-9, previously called Unit III, is less well characterized, but both the 5′ and 3′ junctions have now been cloned (15). The 3′ fragment is 9 to 10 kb in size and has occasionally been mistaken for other fragments of similar size (e.g., the 5′ end of *Mtv-17*).

Mtv-11. This designation was originally used to describe a 5.8-kb *Eco*RI fragment which was thought to represent either a partial provirus or one which lacked an internal *Eco*RI site (24). *Mtv-12* was used to designate a 15-kb *Eco*RI fragment again thought to represent a defective or deleted provirus (24). However, various studies now indicate that the 15.0- and 5.8-kb fragments constitute a single provirus (5, 9, 17, 20). Prakash and colleagues (19) cloned a large 5′ *Eco*RI fragment of C3H/He mice and, using a unique flanking sequence probe, demonstrated that it is located on chromosome 14. Their further suggestion that this fragment is linked with a 5.8-kb 3′ end indicates that the cloned fragment represents the 5′ end of *Mtv-11*, rather than a distinct C3H/He Unit I (16.7-kb) provirus as originally thought.

Mtv-13. The 9-kb *Eco*RI fragment was originally also described as a partial or defective provirus, but at least two studies now show that it is part of a full-length provirus which has been mapped to chromosome 4 (9, 11, 12). The 3′ end of this provirus produces a 5.8-kb fragment. Thus, two proviruses, i.e., *Mtv-11* and *Mtv-13*, both produce a 5.8-kb *Eco*RI junction fragment. This 5.8-kb fragment was originally thought to represent a single defective proviral gene (24).

Mtv-14. This provirus produces a single subgenomic fragment of 1.7 kb. Its chromosomal map location is unknown. The data for its original linkage to chromosome 6 near *Ly-2* by using recombinant inbred lines was not statistically significant (22, 24), and a subsequent attempt to confirm this location with backcross mice was unsuccessful (20).

Mtv-15 and *Mtv-16*. The proviruses assigned these designations are identical to previously identified proviruses.

Mtv-17. A 10-kb *Eco*RI fragment is known to be the 5′ junction associated with a 8.3-kb 3′ end in the locus designated *Mtv-17* on chromosome 4 (5, 9, 11). A 10-kb fragment was previously erroneously thought to represent three proviruses: *Mtv-9* (see above); a partial proviral gene designated *Mtv-15*, now defunct; and the 5′ end of a full-length provirus with an 11.7-kb 3′ end, which was assigned to chromosome 1 as *Mtv-10* (also defunct). The correctly defined 8.3- to 10-kb proviral locus has been referred to as *Mtv-10*, *Mtv-17*, and *Mtv-20* in various publications (5, 11, 16, 18). Here we use the designation *Mtv-17* since this was proposed when the 3′ end of the locus was first molecularly cloned and it avoids the errors associated with the characterization and chromosomal mapping of *Mtv-10*.

Mtv-20. This provirus is found in some, but not all, colonies of C57BL mice and is not carried by C57BL/6 or C57BL/10 mice (25).

Provisional MMTV proviral genes. In addition to the 13 proviruses listed above and in Table 1, a number of studies have described other *Mtv* proviruses for which supporting data is limited to studies of tumor incidence and virus gene expression or to strain comparisons with viral probes. None of these genes has been molecularly cloned, analyzed with probes derived from flanking cellular sequences, or shown to assort independently of known genes in genetic crosses. Therefore, these gene designations are considered provisional, and they have not been included in Table 1.

Mtv-4 refers to a single genetic locus of the inbred Swiss mouse SHN, which controls the production of infectious virus and the occurrence of early mammary tumors (8). Although this locus is phenotypically similar to *Mtv-2*, SHN mice lack the characteristic *Mtv-2 Eco* RI fragments. It has not been determined which of the restriction fragment(s) detected in this mouse comprise *Mtv-4*.

Mtv-5. SL/NiA mice contain a genetic locus designated *Mtv-5* associated with expression of the viral *gag* gene but not with expression of the *env* gene (7). The *Eco*RI fragments associated with expression of this gene have not been identified.

Mtv-18. This designation has been used for two different proviral genes. The first is now known to be identical to *Mtv-3*, and the second was recently identified as a novel 9.2-kb fragment present in the single recombinant inbred strain CxBJ (J. Hilgers and R. Michalides, Mouse Newsl. **70**:63–64, 1984). This fragment is absent from the progenitor strains BALB/c and C57BL/6 and from all other recombinant inbred strains produced from these mice. It may represent a recent germline integration but has not been analyzed further.

Mtv-22 through *Mtv-25* were defined from strain comparisons by the use of viral probes (15). *Mtv-22* is composed of 17.0:kb 5′ and 11.5-kb 3′ fragments and has been detected in AKR and NZB mice. *Mtv-23* is composed of 11.8-kb 5′ and 4.6-kb 3′ fragments and was detected in AKR and A/St mice. *Mtv-24* and *Mtv-25* were found in the European strain DDSio. *Mtv-24* is composed of a 20-kb 5′ and a 6.6-kb 3′ fragment and is also carried by NZB mice. *Mtv-25* is composed of 5.9-kb 5′ and 6.1-kb 3′ fragments.

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